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The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism

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Summary: Recent genetic studies have established that the killer cell immunoglobulin-like receptor (KIR) genomic region displays extensive diversity through variation in gene content and allelic polymorphism within individual KIR genes. It is demonstrated by family segregation analysis, genomic sequencing, and gene order determination that genomic diversity by gene content alone gives rise to more than 20 different KIR haplotypes and at least 40–50 KIR genotypes. In the most reductionist format, KIR haplotypes can be accommodated within one of 10 different prototypes, each with multiple permutations. Our haplotype model considers the KIR haplotype as two separate halves: the centromeric half bordered upstream by KIR3DL3 and downstream by 2DL4, and the telomeric half bordered upstream by 2DL4 and downstream by 3DL2. There are rare KIR haplotypes that do not fit into this model. Recombination, gene duplication, and inversion can however, readily explain these haplotypes. Additional allelic polymorphism imposes extensive individual variability. Accordingly, this segment of the human genome displays a level of diversity similar to the one observed for the human major histocompatibility complex. Recent application of immunogenetic analysis of KIR genes in patient populations implicates these genes as important genetic disease susceptibility factors.

Introduction

Natural killer (NK) cells constitute a lineage of lymphoid effector cells capable of recognizing 'abnormal' cells such as

*KIR gene designation in this paper follows the generally accepted gene designation nomenclature. KIR genes with two extracellular Ig domains are named KIR2D, while KIR genes with three extracellular domains are designated KIR3D. KIR genes containing ITIMs are named 'L' and KIR genes without ITIMs are named 'S'. Accordingly, KIR genes with the 'L' designation are predicted to be inhibitory receptors, while KIR genes with 'S' are predicted to encode activating receptors. In addition, KIR genes with 'S' designation contained within their transmembrane (TM) region a positively charged amino acid, either arginine (R) or lysine (K). The exception to this rule is the KIR2DL4 gene which contains an 'R' in the TM domain and a single ITIM in the cytoplasmic domain. The individual extracellular Ig domains are named D_0 for the most membrane distal domain followed by D_1 and D_2, with D_2 being the membrane proximal domain. The KIR3D receptors contain the D_0, D_1, and D_2 domains. KIR2DL4 and 2DL5 contain D_0-D_2, while all other KIR2D receptors contain D_1 and D_2.

transformed tumor cells, virus infected cells and cells undergoing 'stress'. NK cells mediate an immediate immune response that normally precedes adaptive immune responses, and they contribute to these responses by their secretion of cytokines and chemokines (1, 2). NK cells express a broad array of activating and inhibiting receptors, and their effector function is regulated by a balance between incoming activating and inhibitory signals (3–5). Kärre, Ljunggren and coworkers demonstrated by using tumor models both *in vitro* and *in vivo* that NK cells were activated by loss of self major histocompatibility complex (MHC) class I antigens on the target cells. These observations led to the formulation of the 'missing self' hypothesis, which stated that loss of target cell MHC class I removed the ligands for inhibitory NK receptors, resulting in release of NK cell effector function. According to this model, the inhibitory signals mediated by MHC class I ligand interactions with inhibitory NK receptors dictate NK function (6–8). Recent studies using tumor models expressing ligands for the activating NK receptor NKG2D, such as major histocompatibility class I chain (MIC) related antigens MICA, MICB, Rae-1, and ULBPs, have demonstrated that NK cells can also be activated even when target cells express MHC class I antigens (9–12). It is therefore not currently known exactly how concurrent inhibitory and activating signals coordinate to regulate NK effector function. It is evident that MHC class I antigens and their interactions with inhibitory NK receptors are important for their regulation, but how these inhibitory responses are integrated into the overall NK cell response needs to be determined.

Genetic analysis of NK receptor genes provides indirect evidence for an important role of inhibitory NK receptors and MHC class I ligands in regulation of NK cell function. Initial studies in the mouse identified genes encoding Ly49 receptors with ligand specificity for murine MHC class I molecules (13–16). Subsequently, several groups of investigators identified in the human genes encoding killer cell immunoglobulin-like receptors (KIRs) that displayed ligand specificity for human leukocyte antigen (HLA) class I molecules (17–19). It is now well established that the murine Ly49 gene complex and the human KIR gene complex represent functional analogs (20–23). They are, however, structurally distinct protein families: KIR genes encode Ig-related molecules, while Ly49 genes encode C-type lectin-related proteins. Despite this distinction, both gene families share many features. Subsets of KIR and Ly49 genes encode inhibitory receptors with ligand specificity for MHC class I molecules. These receptors mediate their inhibition by recruitment of cytoplasmic tyrosine phosphatases to the immunoreceptor tyrosine-based inhibitory motif (ITIM) within their cytoplasmic domain. Other KIR and Ly49 genes encode activating receptors that associate with the immunoreceptor tyrosine-based activation motif (ITAM)-bearing molecule DAP12. It is also characteristic for both KIR and Ly49 gene families to contain pairs of activating and inhibitory receptors that display very high nucleotide homology in the extracellular domains (reviewed in 3–5). It should be noted that humans most likely evolved from an ancestral species containing Ly49 genes, since a single Ly49 pseudogene is present within the natural killer complex (NKC) on human chromosome 12 (24, 25). The NKC-syntenic region in the mouse resides on chromosome 6, which also contains the genes encoding other C-type lectin receptors expressed on NK cells such as CD94, NKG2A, -C and -D, in addition to multiple Ly49 genes. The KIR genes are located on human chromosome 19 in a region known as the leukocyte receptor cluster (LRC), which also contains other members of the immunoglobulin (Ig) superfamily, such as the Ig-like transcripts (ILTs), the leukocyte-associated inhibitory receptors (LAIRs), FcαR (CD89), and the natural cytotoxicity receptor NKp46 (23). Like the NKC, the LRC has a syntenic region defined in the mouse, located on chromosome 7. While there are many similarities between the human and mouse LRC, no mouse homologs of human KIR genes have been found (*Fig. 1*).

It has been estimated that the gene duplication events leading to the generation of the KIR genomic region occurred 30–45 million years ago (26). Evidence of one functional inhibitory Ly49 gene and multiple KIR genes in the bovine genome supports the model that the ancestral Ly49 gene predated the divergence of placental mammals and that the ancestral KIR predated primate radiation (27). It is thought that the human KIR genomic region has evolved as a rapidly expanding gene family during the divergence of primates from other mammalian lineages. Comparison of the KIR genomic region within primate species has demonstrated rapid changes estimated to span only a few million years (26–30). Independently and in parallel, the Ly49 family seems to have evolved from an ancestral gene into a multigene family within rodents (31). Analysis of pufferfish, zebra fish and channel catfish has identified a very large family of Ig-like receptor genes, many of which encode cytoplasmic domains with ITIMs. It is not currently known if any of these novel immune-type receptor genes encodes receptors with ligand specificity for MHC class I molecules, but it is intriguing nonetheless to speculate that they may represent the very early ancestors for the LRC genes (32–34).

Therefore, inhibitory NK receptor genes with ligand speci-

ficity for MHC class I molecules are present together with activating receptor genes within a large gene cluster of highly homologous genes that have undergone rapid evolution, both in rodents and in primates. In contrast to what is known for many inhibitory receptors, ligand specificity for activating KIR genes remains obscure. Some studies have suggested they have very weak ligand specificity for MHC class I (35–37), while other studies have failed to demonstrate such interactions (38–41). The recent finding of the murine activating Ly49H receptor demonstrating ligand specificity for murine cytomegalovirus proteins implies a similar functional relationship between activating KIRs and human pathogens (42, 43). While the ligands for activating receptors remain to be determined, it is important to note that many inhibiting KIRs also do not have well-defined ligand specificity.

The emerging interpretation of the function of KIR region genes is that each individual has at least one inhibitory KIR gene with ligand specificity for at least one self HLA class I molecule. It is hypothesized that this provides one of the important regulatory, inhibiting signals needed for tolerance to 'self' and protection against NK-cell-mediated auto-aggression. Other receptor–ligand interactions that are likely to

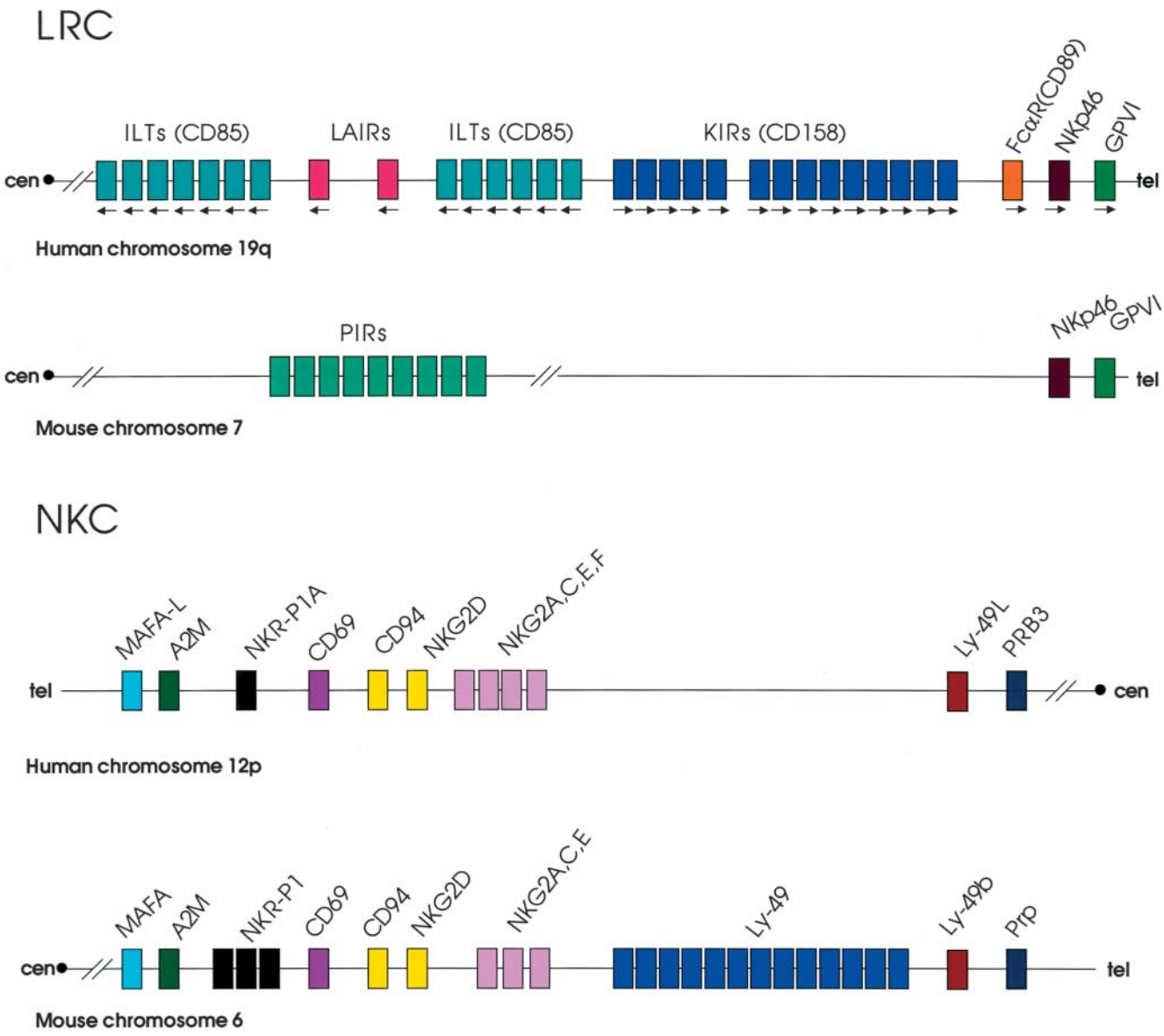

Fig. 1. Genomic organization of NK receptor genes in human and mouse. Top panel: Organization of the leukocyte receptor cluster (LRC) in human (chromosome 19q13.4) and mouse (chromosome 7). Bottom panel: Organization of the natural killer complex (NKC) in human (chromosome 12) and mouse (chromosome 6). The diagrams have been adapted from (20, 21, 23).

contribute to this protective regulation of NK cells include CD94/NKG2A interactions with HLA-E (44, 45) and ILT-2 interactions with HLA-class I (46). In contrast, the activating KIR genes may function by providing NK cell activation in response to pathogens, particularly viral antigens and 'altered' self MHC class I molecules. This model would place KIR genes as important components in regulating NK cell functions by providing a genetic system for tolerance to self and activation of early defense mechanisms against pathogens and transformed cells.

The present review of the KIR genomic region will provide an update on new developments in this area. Recent progress has been made in the genomic analysis of the KIR region: segregation patterns in family studies, nucleotide sequencing of complete and partial KIR haplotypes, and the application of KIR genomic typing to patient populations.

KIR genomic region

The human KIR gene family is located on chromosome 19q13.4 within the LRC. Centromeric to the LRC are the genes encoding Siglecs (sialic-acid-binding-Ig-like lectins), including CD22, the CD66 gene family including the carcinoembryonic antigen (CEA) genes, and the genes encoding the adapter molecules DAP10 and DAP12. Within the LRC but telomeric to the KIR region are the genes encoding FcαR (CD89), NKp46, and platelet glycoprotein receptor VI (GPVI), a platelet collagen receptor (23).

The KIR region itself is at once extremely variable yet highly organized. The KIR genes are arranged in a tightly organized manner, with as many as 14 KIR genes and pseudogenes situated in a head-to-tail fashion, each gene spaced approximately 2.4 kb apart from the other. The one exception to the 2.4 kb-interval pattern is the interval between the pseudogene 3DP1 and KIR2DL4, which measures a sizeable 14 kb*. Further contributing to the organization of the region is the presence of KIR3DL3, the pseudogene 3DP1, 2DL4, and 3DL2, the so-called anchor genes exhibited in their distinctive positions by nearly all haplotypes. Genomic diversity of the KIR region is achieved on several levels. Apart from the anchor genes, KIR gene content varies from individual to individual, such that one haplotype can exhibit from as few as eight to as many as 14 KIR genes and pseudogenes. KIR gene copy number is also variable, with at least two KIR genes known to be exhibited more than once on one haplotype (47, 48). Finally, KIR gene polymorphism figures to be the biggest contributor to diversity of the KIR region, with multiple alleles known and likely more still yet unidentified for each KIR locus. The purpose of such variability presumably is the diversification of the immune response in the context of an environment of rapidly changing pathogens. Characterization of KIR genotypes in different disease populations will help to define the influence of specific KIR genes and their polymorphisms on different disease states such as infection and auto-immunity.

Early population studies of KIR genotypes demonstrated variations in KIR gene content from individual to individual (49–55). Based on these studies, two major KIR haplotype groups, the A and B haplotypes, have emerged (49). The A haplotype has traditionally been defined as containing KIR3DL3, -2DL3, -2DL1, -2DL4, -3DL1, -2DS4 and -3DL2. In contrast, B haplotypes are more variable and characterized by the presence of more than one activating KIR gene.

The existence of A and B haplotypes was further supported by nucleotide sequencing of the complete genomic KIR region. These studies were limited to two reports. The sequences provided in accession number AC011501.7 and analyzed by Martin et al. (56) provide a preliminary description of one representation of a B haplotype. Due to repeated changes in sequence assembly, however, it has been uncertain how this single bacterial artificial chromosome (BAC) clone is composed. Wilson and coworkers (23) described the gene order for both an A haplotype and a B haplotype through a more detailed analysis. These studies, however, did not address the issue of multiple B haplotypes, as had been predicted by the studies of Uhrberg et al. (49). Furthermore, they did not provide information about possible variability within the A haplotype. Recent studies within the last year have addressed both of these issues. In particular, studies of KIR gene segregation within extended families coupled with gene order analyses have resulted in the increased understanding of the organization of the KIR genomic region.

A haplotypes

Population studies using PCR-SSP KIR gene content analysis first identified individuals homozygous for the A haplotype, defining it as containing KIR2DL1, -2DL3, -3DL1, and -3DL2, and estimating its frequency among the Caucasian population to be as much as 47–59% (49). With identification of additional KIR loci, this haplotype was extended to include the putative pseudogene KIR3DL3, a framework locus common to all haplotypes, and KIR2DS4. We have recently shown that traditional typing for KIR2DS4 does not discriminate between the full-length 2DS4 gene and a common gene variant, identical to 2DS4 except for a 22 base-pair deletion in the sequences encoding the second Ig domain (D2) (57). The deletion causes a frame shift in translation, resulting in a

predicted disruption of D2 and premature termination one amino acid within the transmembrane domain. Because of significant amino acid homology and similarities in derivation with the rhesus monkey MmKIR1D gene, the human 2DS4 variant has been termed KIR1D (30). The function of KIR1D is unknown, but its predicted protein structure implies that it is not likely to fulfill the same functions as the intact activating receptors. Indeed, the possibility exists that KIR1D encodes a soluble protein with one intact Ig-like domain. Nevertheless, in the context of the rapid evolution of the KIR genomic region resulting in multiple KIR genes through duplication events, the identification of KIR1D implies a deletion event occurring after the establishment of the 2DS4 gene within the population. It would not be unlikely that such events are occurring within other KIR loci as well, creating altered proteins, thereby effectively eliminating functional KIR proteins from the NK cell surface receptor repertoire.

KIR1D and 2DS4 are alleles to each other, with KIR1D by far the most frequently observed in the Caucasian population (Ag frequency 78.8%, gene frequency 0.54) compared with KIR2DS4 (Ag frequency 35.3%, gene frequency 0.20) (57). The identification of KIR1D refines our understanding of the A haplotype, such that the A haplotype can now be divided into two haplotypes, haplotype A-1D and haplotype A-2DS4, with the former haplotype found at a frequency of 38.8% in Caucasians and the latter haplotype found at a frequency of 11.8% (*Fig.* 2). Indeed, 14% of the population analyzed was homozygous for haplotype A-1D (57). The identification of haplotype A-1D is intriguing because, as the most frequently found haplotype in the Caucasian population, it lacks all functional 2DS-activating KIR receptors. This leaves the central framework gene KIR2DL4 as the sole receptor on this haplotype with activating function, as defined by its ability to mediate cytokine production upon receptor crosslinking (58–60).

B haplotypes

The B haplotypes are a variegated group containing activating KIR loci other than KIR2DS4 or KIR1D. Gene content analysis of families, often multigenerational and with large sibships, has been instrumental in identifying at least 20 different B haplotypes (*Fig.* 2) (47, 48, 57, 61, 62). We and others have applied increasingly specific typing methods to elucidate the B haplotypes, including typing for pseudogenes 2DP1 (47, 57), the two known variants of pseudogene 3DP1 (47, 57), the expressed and nonexpressed variants of 2DL5 (47, 48, 57, 63), and 2DS4 and KIR1D (57). These family segregation studies first demonstrated that the two 2DL5 variants could coexist on the same haplotype (47, 57), a finding that was later confirmed by gene order studies (48).

Linkage disequilibrium (LD) analysis from population genotyping has been useful in defining potential allelic relationships between KIR loci and also suggests common cosegregation patterns of multiple KIR loci (52, 57, 61) (*Table* 1).

From these analyses, several pairs of KIR genes and pseudogenes display negative LD, implying they could be alleles: 2DL3 and 2DL2, 3DS1 and 3DL1, 2DS3 and 2DS5, 2DS1 and 2DS4 and 1D. Indeed, nearly all haplotypes identified thus far support these allelic relationships. Positive LD between locus pairs implies proximity along the chromosome and cosegregation. One of the most striking positive LD relationships is that between 2DS2 and 2DL2, which predicts cosegregation, a finding borne out in identified haplotypes. A surprising finding is the difference in LD profiles between the expressed and nonexpressed subtypes of KIR2DL5. While one might expect that the two are alleles to one another and thus only one or the other occupies the same positional locus, their LD patterns do not fully support this relationship. For example, while the expressed 2DL5 subtype displays strong positive LD with 2DS5 and 2DS3, the nonexpressed 2DL5 subtype displays strong positive LD with 2DS3 and not 2DS5. In addition, the expressed 2DL5 subtype also has positive LD with 3DS1, while the nonexpressed variant displays positive LD with 2DL2 (57).

These LD relationships achieve more clarity upon closer examination of the B haplotypes. Short of sequencing KIR haplotypes, the most useful tool for identifying KIR haplotypes has been gene order mapping. It had previously been shown that the nonexpressed 2DL5 variant could be linked by long-range polymerase chain reaction (PCR) analysis to the 2DL2 gene, while the expressed 2DL5 variant is linked to 3DS1 (63). Recent studies have employed a PCR method to establish gene order on haplotypes even in heterozygous combinations (48). The latter studies have demonstrated the presence of the expressed KIR2DL5 variant in the telomeric half of the haplotype, just downstream to 3DS1. Consistent with the LD analysis, the expressed variant is found associated with either 2DS3 or 2DS5. In contrast, the nonexpressed KIR2DL5 variant is situated in one of two positions: either in the centromeric half of the haplotype, directly downstream to 2DL2 but upstream of pseudogene 2DP1, or, more rarely, in the same position as the expressed variant, downstream to 3DS1. Again, consistent with the LD analysis and in contrast to the expressed variant, the nonexpressed variant appears to

be paired most commonly with KIR2DS3. The presence of both 2DL5 variants in one haplotype, each paired with its respective activating KIR allows for the possibility of having as many as 14 different KIR loci on one haplotype.

Haplotype frequency

Haplotype frequencies in a Caucasian panel of 85 unrelated donors representing 170 haplotypes were determined by haplotype counting (57). Here, haplotypes were assigned to panel donors by first applying the haplotypes defined in family studies and only defining 'new' haplotypes when this approximation was not possible. In this panel, the two different A haplotypes accounted for 86 of 170 haplotypes (frequency 0.506). The most common A haplotype (A-1D) was observed 66 times (frequency 0.388), while the second haplotype (A-2DS4) was seen 20 times (frequency 0.118). The B haplotypes were observed 84 times (frequency 0.494) demonstrating that A and B haplotypes in this unrelated panel of donors occurred with very similar frequency. The B haplotype displayed, however, a much greater diversity, with 12 different haplotypes occurring more than once in the donor panel. Their frequencies varied between 0.124 and 0.012 (*Table* 2).

KIR haplotype model

It is clear that although the potential for KIR diversity based on gene content alone is immense, there are certain patterns that appear conserved within the population. Based on the haplotypes identified thus far, we can develop a model for variations in KIR gene content within haplotypes. In the most reductionist format, KIR haplotypes could be accommodated within one of ten different prototypes, each with multiple permutations.

Our haplotype model considers the KIR haplotype as two separate halves: the centromeric half bordered upstream by KIR3DL3 and comprising those KIR genes upstream of anchor gene KIR2DL4, and the telomeric half bordered downstream by 3DL2 and comprising those KIR genes downstream of 2DL4 (*Fig.* 3). Pairing variants of the centromeric half with variants from the telomeric half encompasses nearly all identified haplotype permutations. There are rare KIR haplotypes,

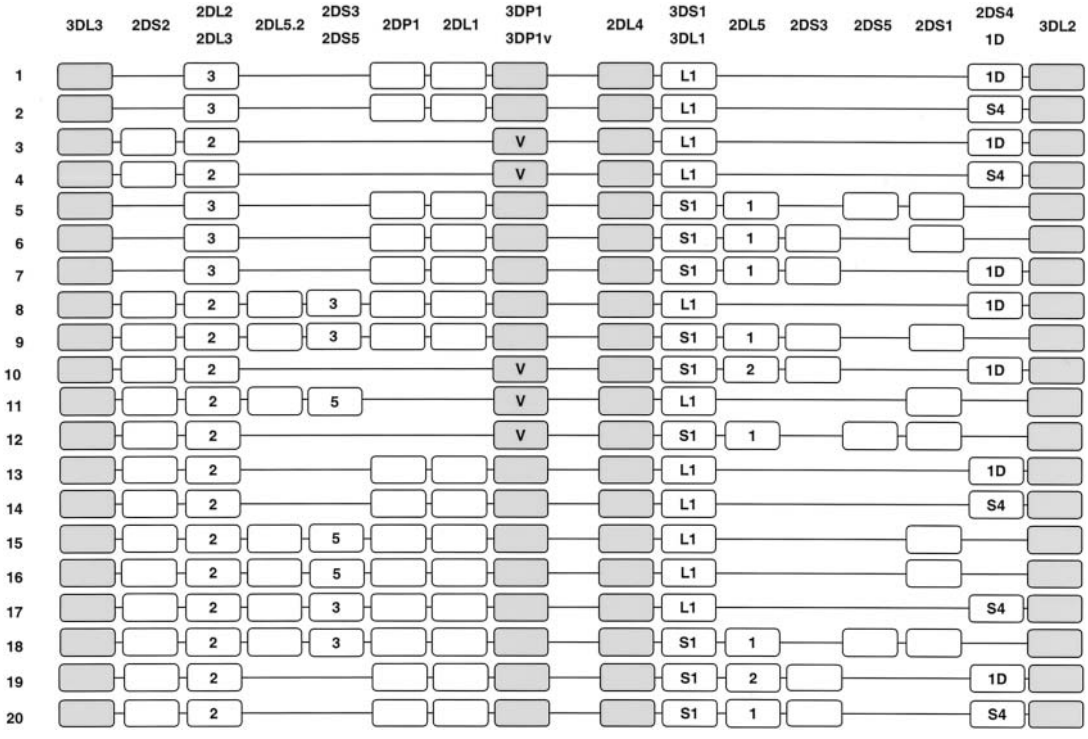

***Fig.* 2. KIR haplotypes defined by family studies and genomic sequencing.** All haplotypes listed have been identified by at least two independent studies. KIR haplotypes defined by family segregation studies were obtained from (47, 57, 61, 62.) KIR haplotypes defined by genomic sequencing and gene-order analysis were obtained from (48). Allele designations for KIR2DL5 are shown within the corresponding box. 1 collectively denotes the expressed 2DL5 variants 2DL5.1 and 2DL5.3 and 2 collectively denotes the non-expressed 2DL5 variants 2Dl5.2 and 2DL5.4. Shaded boxes denote framework KIR genes.

***Table 1*. Genetic linkage disequilibrium analysis for KIR genes. Genetic linkage disequilibrium (LD) analysis for all KIR gene combinations (adapted from 57)**

	2DL2	2DL3	2DP1	2DL1	3DP1	3DP1v	3DL1	3DS1	2DL5.1	2DL5.2	2DS3	2DS5	2DS1	2DS4	1D
2DS2															
Δ	0.22	−0.254	−0.18	−0.194	−0.194	0.109	−0.147	0.031	0.016	0.102	0.077	0.031	0.031	−0.005	−0.067
P	≪0.0005	<0.001	<0.025	<0.025	<0.025	≪0.0005	NS	NS	NS	≪0.0005	≪0.0005	NS	NS	NS	NS
2DL2															
Δ		−0.254	−0.18	−0.194	−0.194	0.109	−0.147	0.031	0.016	0.102	0.077	0.031	0.031	−0.005	−0.067
P		<0.001	<0.025	<0.025	<0.025	≪0.0005	NS	NS	NS	≪0.0005	≪0.0005	NS	NS	NS	NS
2DL3															
Δ			0.166	0.18	0.18	−0.099	0.072	0.026	−0.017	−0.089	0.003	−0.088	−0.094	0.024	0.069
P			≪0.0005	≪0.0005	≪0.0005	<0.005	<0.05	NS	NS	<0.005	NS	<0.025	<0.025	NS	NS
2DP1															
Δ				0.19	0.19	−0.224	0.051	0.018	0.019	−0.027	0.038	−0.062	−0.046	0.03	0.03
P				≪0.0005	≪0.0005	<0.0005	NS	NS	NS	NS	NS	NS	NS	NS	NS
2DL1															
Δ					0.205	−0.242	0.046	0.028	0.019	−0.027	0.041	−0.045	−0.061	0.036	0.021
P					≪0.0005	≪0.0005	NS	NS	NS	NS	NS	NS	NS	NS	NS
3DP1															
Δ						−0.242	0.046	0.028	0.019	−0.027	0.041	−0.045	−0.061	0.036	0.021
P						≪0.0005	NS	NS	NS	NS	NS	NS	NS	NS	NS
3DP1v															
Δ							−0.029	−0.019	−0.03	−0.019	−0.021	−0.006	−0.01	0.018	−0.043
P							NS	NS	NS	NS	NS	NS	NS	NS	NS
3DL1															
Δ								−0.163	−0.168	−0.083	−0.078	−0.067	−0.163	0.043	0.117
P								<0.025	<0.025	<0.001	<0.025	NS	<0.025	NS	≪0.0005
3DS1															
Δ									0.166	0.048	0.081	0.102	0.139	−0.043	−0.058
P									≪0.0005	<0.01	≪0.0005	≪0.0005	≪0.0005	<0.05	NS
2DL5.1															
Δ										0.039	0.061	0.106	0.142	−0.037	−0.07
P										<0.05	≪0.0005	≪0.0005	≪0.0005	NS	<0.05
2DL5.2															
Δ											0.082	0.032	0.048	−0.029	−0.031
P											≪0.0005	<0.05	<0.01	NS	NS
2DS3															
Δ												−0.014	0.03	−0.034	0.011
P												NS	NS	NS	NS
2DS5															
Δ													0.143	−0.043	−0.102
P													≪0.0005	NS	<0.005
2DS1															
Δ														−0.043	−0.102
P														<0.05	<0.01
2DS4															
Δ															−0.153
P															≪0.0005

which do not fit into this model. These haplotypes can, however, readily be explained by recombination, gene duplication and inversion.

Centromeric half

The centromeric half of the haplotype is characterized by the presence of KIR2DL3 or 2DL2, but not both, and, extremely rarely, neither. Where 2DL2 is present, it is always paired with 2DS2, which gene order studies have demonstrated lies directly adjacent to 3DL3. KIR2DL3 defines a partial haplotype, in that it is always paired with the trio of KIR2DP1, -2DL1, and -3DP1. In contrast, while KIR2DS2 and 2DL2 can also be found with 2DP1, -2DL1, and -3DP1, 2DS2 and 2DL2 define a separate partial haplotype, which is characterized by the presence of the exon 2-containing pseudogene variant 3DP1v and by the absence of 2DP1 and 2DL1. The unexpressed 2DL5 variant, when seen in the centromeric half, is paired with 2DL2 and 2DS3 or rarely with 2DL2 and 2DS5.

Telomeric half

Downstream to KIR2DL4, the telomeric half of the KIR haplotype is characterized by the presence of 3DL1 or 3DS1, but not both, or, rarely, neither. The presence of KIR3DL1 indicates a 'short' telomeric segment to follow, containing either 2DS4 or KIR1D and bordered at its most downstream end by 3DL2. In contrast, the presence of KIR3DS1 defines a 'long' telomeric segment, containing a 2DL5 subtype, paired with 2DS3 or 2DS5, followed by a locus occupied by 2DS1, 2DS4, or KIR1D. This locus is then followed by KIR3DL2, which completes the telomeric segment.

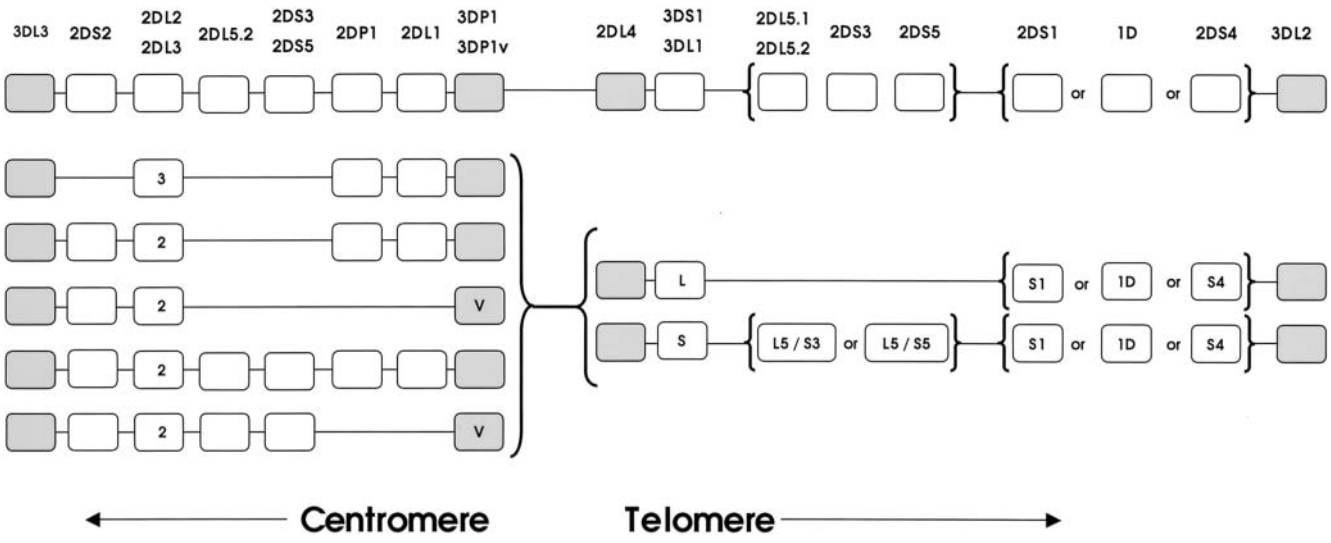

***Fig.* 3. KIR haplotype model.** The centromeric half of the figure indicates five different gene combinations (i.e. centromeric haplotype fragment). The telomeric half of the figure indicates two different gene combinations (i.e. telomeric haplotype fragment). Each centromeric haplotype fragment can combine with any of the two telomeric haplotype fragments, giving a total of 10 prototypic haplotypes. Additional permutations are generated within the telomeric haplotype fragments by combining different combinations of genes from within each bracket.

Genomic sequencing of KIR region and KIR gene order

In order to further our knowledge of the genomic organization at KIR in detail and to uncover new KIR haplotypes, we analyzed a long KIR haplotype found in an individual we had identified as homozygous at KIR due to consanguineous parentage. By sequencing a combination of BACs and long range PCR products derived from LCL WT47 (64), we were able to determine the complete sequence of a long B haplotype in that individual (48). This sequence contained a tandem arrangement of 14 KIR genes centromeric to telomeric: 3DL3/2DS2/2DL2/2DL5.2/2DS3/2DP1/2DL1/3DP1/2DL4/3DS1/2DL5.1/2DS5/2DS1/3DL2 (48). This gene arrangement is essentially similar overall to that reported by Wilson et al. (23), but with some notable exceptions. First, their genomic sequence placed the 2DS2 gene between 2DS1 and 3DL2 at the telomeric end of the complex rather than between 3DL3 and 2DL2 at the centromeric end, as found in our study. A second difference in gene number, with 10 KIR sequences as compared to 14 found in WT47, was consistent with a single deletion event of the 4 genes 2DL5.2 through 2DL1 inclusive from the WT47 haplotype. From further exhaustive genomic analysis, we predict that the haplotype containing 14 KIR sequences in tandem and represented in LCL WT47 represents the longest KIR haplotype in the human population.

We had chosen to focus our sequence assembly on the longest KIR haplotype as a starting point in the establishment of genomic data useful for the ultimate goal of genotyping the KIR locus. With this goal in mind, we developed two types of SSP reactions: one to identify the presence or absence of specific KIR loci, and a second to establish the adjacency of individual loci. Gene-specific KIR-SSPs were developed by choosing sites within exon sequences that were invariant in the available cDNA sequences. Order-specific reactions were developed by finding unique sequences located within the 3′ ends of genomic loci and pairing them with sequences from the 5′ ends of adjacent loci within our established genomic

***Table* 2. KIR haplotype frequencies. KIR haplotypes were deduced in a panel of 85 Caucasoid donors, representing 170 haplotypes (adapted from 57). Haplotype frequencies are given where they occured more than once among the panel donors. Haplotypes occurring only once are grouped together as 'others'**

Haplotype	*n* = 170	Frequency
A Haplotype (Total)	86	0.506
#1	66	0.388
#2	20	0.118
B Haplotype (Total)	84	0.494
#5	21	0.124
#3	13	0.077
#4	11	0.065
#8	7	0.041
#9	6	0.035
#6	4	0.024
#14	4	0.024
#17	4	0.024
#13	3	0.018
#19	3	0.018
#11	2	0.012
#20	2	0.012
Others	4	0.024

sequence. For KIR loci that were not contained within the KIR genomic sequence determined in this report, we relied on KIR cDNA and genomic fragments deposited in GenBank.

For some genes it was possible to further discriminate between two groups of allelic variants. For example, 3DL1 was divided by reactions specific for 3DL1.1 and 3DL1.2 using a conserved 3DL1 specific base in exon 5 in combination with either of two residues that were found split between all 3DL1 allelic variants. Similarly, the 2DL4A and 2DL4B SSPs divided this gene into two broad allelic groups. As discussed earlier, the allelic discrimination for the 2DS4 gene (2DS4 vs KIR1D) may be of more significant physiological importance. For the 2DL5.1 and 2DL5.2 genes, three reactions were designed, one that detects the presence of either gene using exon sequences and two others that discriminate the genes in the intron sequence.

Order specific SSPs were chosen, so that in theory any KIR locus could be tested for adjacency with any other locus by combining the 5′ or 3′ specific primer from one gene with the 3′ or 5′ specific primer from any other gene. Indeed, such an arrangement should also be useful to amplify any locus between genes using long-range PCR. In practice it was not always possible to choose 5′ and 3′ specific primers that could uniquely discriminate all genes, as some of the genes are identical in the 3′ and 5′ regions (exemplified by the 3′ ends of 2DL2 and 2DS2 or the 5′ ends of 2DS3 and 2DS5). To accommodate more precise discrimination when only single base differences were available for specificity, some of the developed SSPs included typing ambiguities. Presently, these typing tools can discriminate all of the known KIR haplotypes and are being refined further through continued application and testing. Using order-specific KIR-SSPs, we were able to define seven new haplotypes (48) in addition to the previously described A and B haplotypes (49). Some key observations made from family segregation analysis and gene order studies were the following:

2DS2 was always identified between 3DL3 and 2DL2 at the centromeric end of the KIR region.

Haplotypes with two 2DL5 genes always have one 2DL5 gene next to 2DL2 and one telomeric of 3DS1. The centromeric 2DL5 gene is commonly linked to 2DS3 and rarely with 2DS5, while the telomeric 2DL5 gene is linked to 2DS3 or 2DS5.

Haplotypes containing 2DL3 and 2DL5 centromeric of 2DL4 have not been identified.

Haplotypes with 2DL2 exist in two forms, one with 2DP1-2DL1-3DP1 and one with 3DP1v without 2DP1-2DL1 (57). Haplotypes with 2DL3 also contain 2DP1-2DL1-3DP1, while 3DP1v has not been found on 2DL3 haplotypes.

Allelic polymorphism of KIR genes

A significant contributor to the diversity of the KIR genomic region is allelic polymorphism (50, 61, 65–71). Generated by point mutation and homologous recombination (50, 61, 65–72), allelic polymorphism has been identified for all KIR loci. While the extent of KIR allelic diversity appears less than for the MHC, it is clear that the limited number of known KIR alleles significantly expands KIR genomic diversity beyond KIR gene content (73). Indeed, high resolution genotyping for polymorphic allelic variants of four loci — KIR2DL1, -2DL3, -3DL1, and -3DL2 — identifies 22 distinct A haplotypes alone, revealing a new dimension to KIR haplotypes beyond gene content, and implying that significantly less than 0.24% of unrelated individuals can be expected to have allotypically identical genotypes (61).

Functional relevance of KIR polymorphism remains largely unclear. Sequence polymorphism of the KIR2DL5 promoter has been found to result in profound differences in 2DL5 expression, possibly by affecting the binding of transcription factor AML1 (63). Sequence variation within the coding regions of the KIR genes, however, has not yet revealed functional consequences. The areas of greatest polymorphism may be some indication of these consequences. Although the putative class I contact residues of the inhibitory KIR are well-conserved within KIR gene families (74, 75), the frequency of polymorphic variation residing in the loops of the Ig-like domains suggests a selection for influencing interaction with potential ligands.

KIR and disease

The extent of the diversity of the KIR genomic region is becoming clearer; however, the advantages of generating such diversity are not obvious. After all, of the four framework loci found nearly ubiquitously in the population, 3DL3, 3DP1, 2DL4, and 3DL2, one is a pseudogene and one is not expressed (76). Non-expressed variants of 2DL5 are nearly as common as expressed variants (gene frequencies 0.28 and 0.43, respectively) (63), and several 3DL1 allotypes demonstrate low to no expression based on antibody binding (66). Furthermore, the most common haplotype within all populations studied is haplotype A, the haplotype with the least number of KIR genes, in particular the activating KIR genes (57). Clues to the importance of KIR diversity and to the function of these genes might be found in the mouse, whose orthologous Ly49 genes evolved in parallel with the KIR genes to perform similar immune functions.

Early reports that the NK cell activation receptor Ly-49H is vitally involved in resistance to murine cytomegalovirus *in vivo* introduced the concept that an activating NK cell receptor could play a critical role in providing innate defense against a viral pathogen (42). Subsequent identification of the ligand for Ly-49H as a murine cytomegalovirus protein (m157) confirmed this role (43). The most intriguing feature of this protein is its predicted three-dimensional structure as an MHC-like protein, suggesting that the NK cell activating receptor may have been selected to counteract the viral device of MHC-mimicry.

Whether similar foreign proteins exist as ligands for human activating KIR remains to be determined. Meanwhile, epidemiological studies of KIR receptors and disease states are contributing to the understanding of the role of the activating KIRs. There is evidence that the combination of activating KIR3DS1 with HLA-B alleles containing isoleucine at position 80 (Bw4-80Ile) is associated with delayed progression to acquired immunodeficiency syndrome (AIDS) in persons infected with human immunodeficiency virus type 1 (HIV-1), and this study would seem to suggest that the Bw4-80Ile molecule behaves as a ligand for KIR3DS1 (77). These findings are consistent with earlier analysis linking control of HIV-1 viremia and protection from progression to AIDS with HLA-Bw4 homozygosity (78). Epidemiological studies involving KIR and autoimmunity have revealed an association between the presence of the activating KIR2DS2 on $CD4^{+}CD28^{null}$ lymphocytes with the development of rheumatoid vasculitis (79). Similarly, both the activating KIR2DS1 and 2DS2 receptors have been associated with psoriatic arthritis, an inflammatory arthritis associated with psoriasis (80). Interestingly, the latter disease association was identified only when the HLA ligands for the inhibitory KIR2DL1, -2DL2, and -2DL3 were absent. These studies together seem to relate the influence of activating KIR with HLA, either by specific HLA-recognition or by specific HLA-absence. These observations are consistent with a model that assumes HLA class I specificity of activating KIR receptors, which may be allowed to mediate NK activation in the absence of down-regulation of inhibitory KIR receptor HLA ligands. To further clarify the balance of influence between the inhibitory and activating KIR in the context of disease pathogenesis, continued epidemiological analysis of KIR and disease should be pursued.

KIR and BMT

Unrelated bone marrow transplant (BMT) from HLA class I and class II genotypically matched donors has been facilitated for recipients who lack a suitably matched related donor (81). Many extensive studies have been conducted showing that donor-recipient identity for human leukocyte antigen alleles apparently reduces the risk of acute graft vs. host disease (GVHD) and improves survival after unrelated BMT. However, in a small subset of these patients, graft rejection occurs for reasons that have an as yet unresolved and likely genetic basis. NK cells have long been implicated in marrow transplant rejection in the mouse, where genetic differences controlling NK reactively are likely causative for graft rejection (82). In humans, recent evidence suggests that mismatch at NK KIR ligands between host and donor tissue was associated with a significantly reduced risk of relapse in acute myeloid leukemia patients (83). It seems that this genetic mismatching not only had a graft vs. leukemia effect but also did not contribute to graft vs. host or to graft rejection. This latter result may be revised by recent work in our laboratories where we studied the association between KIR mismatching and unrelated bone marrow transplant graft rejection (84).

In our work on KIR involvement in marrow transplant, we designed a case–control study using unrelated BMT pairs that had undergone transplants and had failed due to graft rejection. Thirty-one unrelated pairs that had experienced rejection were used as case samples, and 62 unrelated pairs, which did not experience rejection, were used as control samples with prior informed consent. All of these DNAs were HLA typed at class I (HLA-A, -B and -C) and class II (HLA-DP, -DQ and -DR) at high resolution and all patient and control samples were obtained from those receiving treatment for chronic myeloid leukemia (CML). Based upon the data obtained by examining these 174 samples with our SSP KIR typing system, a conditional logistic regression model was applied to test whether or not the distributions of KIR haplotypes were deviated between case and control pairs. Those analyses showed significant probability that the number of nonmatched pairs of KIR haplotypes were increased in case pairs as compared to that of control pairs. Thus we propose that by considering the whole effect of KIR haplotypes, not only an individual effect from each KIR gene, greater statistical power and association might be achieved in genetic studies. By combining this strategy with HLA genotyping, further additional statistical power might be obtained towards understanding the genetics underlying a variety of HLA associated diseases.

Concluding remarks

The Ig-like receptor genes of the KIR family have evolved as a rapidly expanding gene family, which displays a very high

degree of diversity among individuals. While the ligand specificities for most of these genes still are unknown, it is highly likely that these receptors play an important role in both innate and adaptive immune responses. Preliminary studies of KIR genes as genetic susceptibility factors for autoimmune and infectious diseases and the possible interactions between their gene products and MHC class I alloantigens points towards very interesting mechanistic interpretations of pathophysiological processes. There is also increasing evidence that NK cells play important functions following allogeneic hematopoietic stem cell transplantation and KIR compatibility or incompatibility could be involved in determining the fate of the graft, protection against leukemia relapse and post-transplantation infectious complications. Epidemiological studies combining histocompatibility testing and KIR genomic typing might also provide important insights on disease susceptibility within different populations and ethnic groups. Together these developments bring forward the KIR gene family as a highly polymorphic immune complex with a strong emphasis on haplotypes as a focus for genetic association studies. The recent development of rapid and precise genomic typing methods for KIR genes and KIR haplotypes will undoubtedly facilitate implementation of many new investigations.

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